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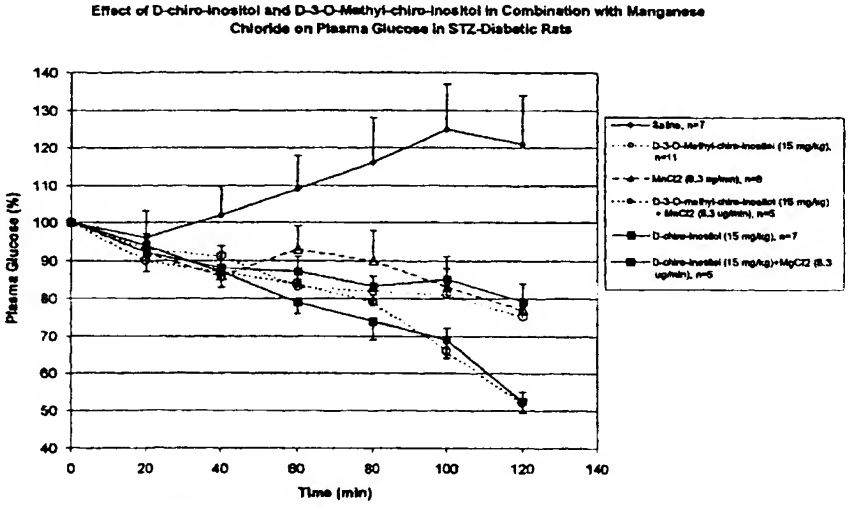

At 120 minutes, the hypoglycaemic effects produced by combination of either D-chiro-inositol or D-3-O-methyl-chiro-inositol with manganese chloride are significantly ($p<0.05$) greater than those produced by D-chiro-inositol or D-3-O-methyl-chiro-inositol alone.

(57) Abstract

The current invention relates to compositions and methods for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. The compositions of the present invention contain (i) a source of a polyvalent metal ion, such as magnesium, manganese, chromium, vanadium and zinc, and (ii) an inositol, such as D–*chiro*–inositol, or a derivative or metabolite of an inositol, such as D–3–*O*–methyl–*chiro*–inositol.

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COMPOSITIONS AND METHODS FOR IMPROVING INSULIN SENSITIVITY AND GLUCOSE METABOLISM IN MAMMALS

FIELD OF THE INVENTION

The current invention relates to compositions and methods for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. The compositions of the present invention contain (i) a source of a polyvalent metal ion, and (ii) an inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol.

BACKGROUND OF THE INVENTION

A. INSULIN AND METABOLISM

In the mammalian body, insulin acts as a kind of spark plug to the mechanism that allows glucose in the blood to enter a cell, where it can be used as energy. Without insulin, sugar levels in the blood increase to dangerous and even life-threatening levels, a condition known in humans as diabetes mellitus. The supply of glucose to the cells is essential not only for energy, but also for the functioning of the mammalian brain and the survival of sensitive nerve tissue.

After food is consumed by a mammal, the pancreas secretes insulin into the bloodstream to maintain an optimal blood sugar level, generally about 4-8 mmol/L (about 72-144 mg/dL) in healthy humans. Other hormones, however, including glucagon, cortisone, adrenaline and growth hormone, act against insulin to prevent the blood sugar level from dropping too low.

When there is insufficient food-derived glucose in the blood to satisfy the needs of the mammalian brain, the body produces its own glucose, first through the breakdown of stored glycogen by the liver and then through gluconeogenesis. In gluconeogenesis, amino acids from food or muscle tissue are converted to glucose. Gluconeogenesis is inhibited by insulin.

A second source of fuel for the mammalian brain is ketone bodies, which are formed from fatty acids stored in adipose tissue. After an extended period of starvation or carbohydrate restriction, ketone bodies are formed by the body (ketogenesis) to supplement the glucose formed by the body through gluconeogenesis. Ketogenesis requires the influence of glucagon to proceed and is inhibited by insulin.

In addition to gluconeogenesis and ketogenesis, insulin also affects a number of other processes in the body. For example, insulin causes a buildup of triglycerides, the storage form of

fatty acids, and inhibits their release from fat cells. Insulin also increases the formation of cholesterol in the liver and similar steroid hormones in the adrenals and gonads. Moreover, insulin favors the retention of water and salt by the mammalian body, thereby increasing blood pressure and aggravating hypertension.

B. INSULIN SENSITIVITY AND ABNORMAL GLUCOSE TOLERANCE

Insulin sensitivity is a measurement of insulin's ability to produce a biological response; specifically, in the case of glucose regulation, insulin sensitivity is a measurement of insulin's ability to promote the clearance and utilization of glucose.

A decrease in insulin sensitivity will result in a prolonged elevation of glucose levels and the release of additional insulin to try and achieve a euglycemic state. This compensatory hyperinsulinemia will effect insulin's ability to suppress lipolysis in adipose tissue, thus causing an increase in free fatty acids and ultimately resulting in the disruption of normal lipid profiles which could lead to coronary artery disease. The increase in free fatty acids will also inhibit insulin-stimulated glucose utilization in the muscle and stimulate hepatic gluconeogenesis. This leads to increased blood glucose and will eventually result in the development of impaired glucose tolerance or impaired fasting glucose and ultimately, if unchecked, the development of Type 2 diabetes. Improving insulin sensitivity will restore overall glucose control and decrease the risk of cardiovascular disease.

In mammals, a reduction in insulin sensitivity (an increase in insulin resistance) is accompanied by an increase in serum glucose concentration. This increase in serum glucose concentration, in turn, causes an increase in insulin production by the pancreas in an effort to maintain normal blood glucose levels. Prolonged elevated serum insulin and glucose levels are therefore characteristic of insulin resistance.

Even though serum glucose levels remain high in insulin resistant mammals due to the high amounts of ineffective insulin, this insulin continues to function normally with respect to its other physiological roles. Thus, an insulin resistant person who already has high blood sugar levels, which normally should signal satisfaction, often experiences carbohydrate cravings, particularly for simple sugars. As more carbohydrates are consumed, the pancreas produces additional insulin

to maintain a constant serum glucose concentration. That additional insulin, however, enhances the insulin resistance (decreases insulin sensitivity) by decreasing the number of available insulin receptors on the surfaces of the cells.

Abnormal glucose tolerance refers to metabolic stages intermediary to normal glucose homeostasis and Type 2 diabetes; this includes conditions like impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) where glucose values are above the conventional normal range and are often accompanied by a decrease in insulin sensitivity. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are transient, intermediate stages in the development of Type 2 diabetes. Within ten-years of diagnosis, approximately 30% of IGT subjects will progress to Type 2 diabetes and potentially to health problems that accompany this disease, including retinopathy, nephropathy, and peripheral neuropathy. In addition, abnormal glucose tolerance and decreased insulin sensitivity are associated with a high risk for the development of hypertension, dyslipidemia and an increase incidence of coronary artery disease.

Abnormal glucose tolerance and decreased insulin sensitivity can be attributed to a wide range of causes including obesity, age, physical activity level, certain medication or drugs, genetic factors, and some endocrine related disorders. The truncal distribution of weight as determined by a high waist to hip ratio (WHR) is a good predictor of abnormal insulin sensitivity, and there is an excellent correlation between a high body mass index (BMI) and decreased insulin sensitivity. Approximately 33% of the population in the United States is obese and the majority of these individuals have decreased insulin sensitivity, are hyperinsulinemic, and often have abnormal glucose tolerance.

Impaired fasting glucose (IFG) is defined by the American Diabetes Association as a fasting blood glucose in the range of 110 mg/dL to 125 mg/dL. IFG is determined by analysis of a plasma sample for glucose after a 10-16 hour fast. This is an easy and quick way to determine if there is a problem with glucose tolerance and metabolism.

Impaired glucose tolerance, as defined by the World Health Organization, is determined by the administration of a standard oral glucose tolerance test (OGTT) (World Health Org., Diabetes Mellitus, Tech. Rep. Ser., no. 727 (1985)). During an OGTT, a measured amount of glucose (75 grams) is given to a fasted individual and blood glucose levels are measured every 30 minutes.

usually for 2 or 3 hours. In a individual with normal glucose tolerance, the blood glucose values will rise during the first part of the test and then rapidly return to basal levels. In an IGT individual the post prandial glucose levels will rise above the normal range, producing a 2-hour glucose value of 140-199 mg/dL, and return to basal levels at a slow rate.

Abnormal glucose tolerance is caused in part by inadequate utilization of glucose in the periphery - at the site of the muscles. In addition, high fasting glucose values, seen with impaired fasting glucose, suggest that hepatic glucose production is not being effectively regulated. The underlying cause of this abnormal glucose tolerance is characterized by a decrease in insulin sensitivity.

Improving insulin sensitivity and glucose tolerance will help delay and even prevent the onset of Type 2 diabetes mellitus, and thus improve the quality of life by preventing acute and long-term complications, reducing mortality and treating accompanying disorders of those at risk for Type 2 diabetes.

Accordingly, there is a need for compositions and methods for improving insulin sensitivity and glucose metabolism in mammals, particularly in human.

SUMMARY OF THE INVENTION

Administration of salts or complexes of certain polyvalent metal ions to mammalian subjects with disease states characterized by reduced insulin sensitivity and/or abnormal glucose tolerance has been shown to produce beneficial therapeutic effects on glucose metabolism. Similarly, administration of certain inositols has also been shown to produce beneficial therapeutic effects on glucose metabolism in such subjects.

It has now been found that certain combinations of: (i) a salt or complex of polyvalent metal ions; and (ii) an inositol or a derivative of inositol have been found to exert significant effects on mammalian endocrinology and metabolism. More specifically, it has now been found that administration of a combination of:

(i) a salt or complex of certain polyvalent metal ion, such as magnesium, manganese, chromium, vanadium and zinc; and

(ii) an isomer of inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol,

to mammalian subjects with reduced insulin sensitivity and/or abnormal glucose tolerance, including type 2 diabetics, significantly improves glucose metabolism and/or increases insulin sensitivity at a lower dose and/or to a greater degree than either the polyvalent metal ion or the inositol alone.

Accordingly, the first embodiment of the present invention is directed to a pharmaceutical composition for improving glucose metabolism and/or increasing insulin sensitivity in a mammal, the inventive composition comprising: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol or a derivative or metabolite of an inositol.

A second embodiment of the present invention is directed to a method for improving glucose metabolism and/or increasing insulin sensitivity in a mammal, which comprises the step of administering to a mammal: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol.

A third embodiment of the present invention is directed to a method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity, which comprises the step of administering to a mammal: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 is a graph comparing the plasma glucose levels of diabetic rats (streptozotocin 45 mg/kg) treated with: (i) NaCl (—◆—, n=7); (ii) 15 mg/kg D-3-*O*-methyl-*chiro*-inositol (red --○--, n=11); (iii) 8.3 µg/min manganese chloride (red --△--, n=8); (iv) 15 mg/kg D-3-*O*-methyl-*chiro*-inositol plus 8.3 µg/min manganese chloride (--○--, n=5); (v) 15 mg/kg D-*chiro*-inositol (red —■—, n=7); and (vi) 15 mg/kg D-*chiro*-inositol plus 8.3 µg/min manganese chloride (—■—, n=5).

The data show that after 120 minutes, the hypoglycaemic effects produced by the combination of a source of polyvalent metal ions (manganese chloride) and either an inositol (D-*chiro*-inositol) or an inositol derivative (D-3-*O*-methyl-*chiro*-inositol) are significantly ($p<0.05$) greater than the effects produced by any of these agents alone.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In a first preferred embodiment, the present invention is directed to compositions for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. These mammals may have diabetes, particularly type 2 diabetes (insulin resistant diabetes), or may have only impaired glucose tolerance and have not yet developed diabetes.

In a second preferred embodiment, the present invention is directed to a method for improving glucose metabolism and/or increasing insulin sensitivity in mammals, particularly humans. These mammals may have diabetes, particularly type 2 diabetes (insulin resistant diabetes), or may have only impaired glucose tolerance and have not yet developed diabetes.

In a third preferred embodiment, the present invention is directed to a method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity. These mammalian metabolic diseases include, but are not limited to, the following: diabetes mellitus and its chronic complications; gestational diabetes; pre-eclampsia; obesity; hyperlipidemia and/or dyslipidemia; atherosclerosis; hypertension; cardiovascular disease; AIDS; cancer; wasting and/or cachexia; sepsis; trauma, such as associated with burns, malnutrition and/or stress; aging; autoimmune diseases, such as lupus; endocrine diseases; hyperuricemia; polycystic ovary disease; and complications arising from athletic activity or inactivity.

The compositions of the present invention, which are particularly useful in the therapeutic methods of the present invention, comprise: (i) an effective amount of a source of polyvalent metal ions; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol. The inventive compositions may also optionally contain one or more pharmaceutically acceptable carriers, diluents and/or excipients.

The polyvalent metal may be any of the known metal elements which have at least one valence or oxidation state of 2 or greater. Thus, the free ions of these polyvalent metals have a positive charge of 2 or more. Illustrative examples of suitable polyvalent metals include, but are not limited to, the following metals: manganese; magnesium; chromium; zinc; vanadium; copper; titanium; and calcium.

Preferably, the polyvalent metal is a divalent metal, *i.e.*, has a valence or oxidation state of 2. Illustrative examples of suitable divalent metals include, but are not limited to, the following: manganese, magnesium, chromium, vanadium and zinc. Some polyvalent metals, such as chromium, exhibit more than one valence or oxidation state. In such instances, it is preferred that the polyvalent metal be used in its divalent state.

The source of polyvalent metal ions in the inventive compositions may be any material that will liberate the desired free polyvalent metal ions in the desired environment, such as in a mammalian body. Suitable sources of polyvalent metal ions include, but are not limited to, the following: polyvalent metal salts, such as magnesium chloride or manganese sulfate; and polyvalent metal chelates, such as magnesium EDTA (ethylene diamine tetraacetic acid).

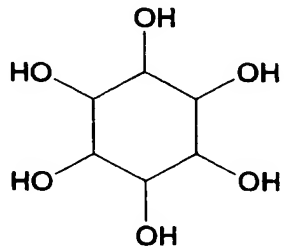
Preferably, the source of polyvalent metal ions is a salt of the desired polyvalent metal and one or more organic or inorganic counterions. Illustrative examples of suitable organic counterions include, but are not limited to, the following: maleate; acetate; tartrate; bitartrate; citrate; oxalate; succinate; benzoate; fumarate; malate; and mandelate. Illustrative examples of suitable inorganic counterions include, but are not limited to, the following: fluoride; chloride; bromide; iodide; sulfite; sulfate; sulfamate; nitrite; nitrate; phosphite; and phosphate.

Particularly preferred counterions are inorganic counterions; more particularly preferred counterions are halides, such as fluoride and chloride; most preferably, the counterion is chloride. Most preferably, the polyvalent metal ion is magnesium or manganese and the source of polyvalent metal ions is the chloride salt thereof.

Optionally, the inventive composition may contain more than one source of polyvalent metal ions. For example, a composition according to the present invention may include both magnesium chloride and magnesium sulfate in admixture.

Additionally, the inventive composition may optionally contain sources of more than one polyvalent metal ion. For example, a composition according to the present invention may include both magnesium chloride and manganese chloride in admixture.

The inositol employed in the inventive compositions may be any of the known isomers of inositol, a carbocyclic sugar having the general formula:



Illustrative examples of suitable isomers of inositol include, but are not limited to, the following: *myo*-inositol and *chiro*-inositol. Preferably, the isomer of inositol is *chiro*-inositol, more preferably D-*chiro*-inositol.

As used herein, a "derivative or metabolite of an inositol" may be any compound based on or derived from or containing a D-*chiro*-inositol moiety. Illustrative examples of suitable derivatives and metabolites of D-*chiro*-inositol include, but are not limited to, the following: D-*chiro*-inositol phosphates; D-*chiro*-inositol esters, preferably acetates; D-*chiro*-inositol ethers, preferably lower alkyl ethers; D-*chiro*-inositol acetals; D-*chiro*-inositol ketals; and compounds containing D-*chiro*-inositol.

As used herein, a "compound containing D-*chiro*-inositol" may be any compound that contains the D-*chiro*-inositol moiety. Illustrative examples of compounds containing D-*chiro*-inositol include, but are not limited to, the following: polysaccharides containing D-*chiro*-inositol and one or more additional sugars, such as glucose, galactose and mannose, or derivatives thereof, such as glucosamine, galactosamine and mannitol; D-*chiro*-inositol phospholipids; and complexes or chelates of D-*chiro*-inositol with one or more metal ions and the like.

Optionally, the inventive composition may contain both an inositol and a derivative or metabolite of an inositol. For example, a composition according to the present invention may include both D-*chiro*-inositol and D-3-*O*-methyl-*chiro*-inositol in admixture.

The compositions of the present invention will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the active agent), the site of delivery of the composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The"effective amount" of each active agent (*i.e.* (i) a source of polyvalent metal ions, and (ii) an inositol, or a derivative or metabolite thereof) for the purposes of the present invention is determined in view of such considerations. Those skilled in the art can readily determine empirically an appropriate "effective amount" of each active agent for a particular mammalian patient.

The key factor in selecting an appropriate dose is, of course, the desired result obtained in terms of improving glucose metabolism and/or increasing sensitivity. These desired results may be measured, for example, by increases or decreases in blood glucose levels and/or insulin sensitivity in the patient. The length of treatment needed to observe changes and the interval following treatment for responses to occur may vary depending on the desired effect and the particular patient, but may be determined empirically by those skilled in the art.

As a general proposition, the total effective amount of each active agent administered per dose will be in the range of about 0.1 μg/kg/day to 100 mg/kg/day of mammalian patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, for humans, the amount per dose is between about 10 μg/day and 2 g/day.

For example, when administered orally, the inventive composition preferably contains from about 1 mg to about 1200 mg of the inositol, or the derivative or metabolite of an inositol. In the case where the inositol is D-*chiro*-inositol, the inventive composition preferably contains from about 10 mg to about 900 mg of DCI, more preferably about 30 mg to about 600 mg of DCI, and most preferably about 100 mg to about 300 mg of DCI. In the case where the derivative or metabolite of inositol is D-3-*O*-methyl-*chiro*-inositol, the inventive composition preferably contains from about 10 mg to about 900 mg of D-3-*O*-methyl-*chiro*-inositol, more preferably about 30 mg to about 600 mg of D-3-*O*-methyl-*chiro*-inositol, and most preferably about 100 mg to about 300 mg of D-3-*O*-methyl-*chiro*-inositol.

As used herein, the phrase "pharmaceutically acceptable" is intended to refer to those

compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, a "pharmaceutically acceptable carrier" is a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the active agents of the inventive compositions from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Some illustrative examples of materials which can serve as pharmaceutically-acceptable carriers include, but are not limited to, the following: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the inventive pharmaceutical compositions.

Illustrative examples of pharmaceutically acceptable antioxidants include, but are not limited to, the following: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble

antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA),butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredients which can be combined with a carrier material to produce a single dosage form will generally be that amount of each active ingredient which, together, produce the desired therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredients, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association at least one source of polyvalent metal ions and at least one inositol (or derivative or metabolite of an inositol) with the carrier and, optionally, one or more accessory ingredients.

In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients the inventive compositions with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of each active ingredient. The active ingredients of the inventive compositions may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredients are mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents.

In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding the active ingredients and carrier material(s), optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredients moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes

and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredients can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the inventive compositions include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredients, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active ingredients, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing the active ingredients of the present invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the

rectum or vaginal cavity and release the active ingredients. Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of the inventive compositions include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredients may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to the active ingredients, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active ingredients, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active ingredients in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredients compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredients in a polymer matrix or gel. Devices, including patches, which transdermally deliver the active ingredients by ionophoresis or other electrically-assisted methods can also be employed in the present invention, including, for example, the devices described in U.S. Patent Nos. 4,708,716 and 5,372,579.

Ophthalmic formulations, eye ointments, powders, solutions, drops, sprays and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise at least one source of polyvalent metal ions and at least one inositol (or derivative or metabolite thereof) in combination with one or more pharmaceutically-acceptable sterile isotonic

aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Illustrative examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include, but are not limited to, the following: water; ethanol; polyols, such as glycerol, propylene glycol, polyethylene glycol, and the like, and suitable mixtures thereof; vegetable oils, such as olive oil; and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorbutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject active ingredients in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and

poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.01 to 99.5% (more preferably, 0.1 to 90%) of each active ingredient together in combination with at least one pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc.; administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is particularly preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein are intended to mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein are intended to mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The inventive compositions may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the active ingredients of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions

of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

As noted, actual dosage levels of the active ingredients in the inventive pharmaceutical compositions may be varied so as to obtain an amount of each active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors, including, but not limited to, the following: the activity of the particular polyvalent metal ions and the particular inositol (or derivative or metabolite) employed; the route of administration; the time of administration; the rates of absorption, distribution, metabolism and/or excretion of the particular active ingredients being employed; the duration of the treatment; other drugs, compounds and/or materials used in combination with the particular active ingredients employed; the age, sex, weight, condition, general health and prior medical history of the patient being treated; and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine the effective amount of the each active ingredient required in the inventive pharmaceutical compositions. For example, the physician or veterinarian could start doses of the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

If desired, the effective daily dose of the active ingredients may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for the active ingredients of the present invention to be administered alone, it is preferable to administer these compounds as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, a therapeutic composition of the present invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824, or 4,596,556. Examples of well-known

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implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

The following examples are illustrative only and are not intended to limit the scope of the invention as defined by the appended claims. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit and scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

All patents and publications referred to herein are hereby expressly incorporated in their entirety by reference.

EXAMPLES

Materials and methods

The streptozotocin (STZ) treated rat is a widely accepted and frequently used animal model of diabetes mellitus. At low doses of STZ, rats develop mild basal hyperglycemia, glucose intolerance and impaired glucose-induced insulin secretion. Therefore, this model appears to be an excellent model of human type 2 (non-insulin dependent) diabetes and well-suited for the study of novel antidiabetic agents (*see* Pele-Tounian *et al.*, *British J. Pharmacol. 124*:1591-1596 (1998)). At larger doses of STZ, absolute insulinopenia and extreme levels of glycemia develop, and the diabetes becomes life-threatening similar to human type 1 (juvenile onset) diabetes.

In addition to being an excellent model of aberrant insulin and glucose metabolism in humans, the STZ-treated diabetic rat is a widely used model of the severe and life-threatening long-term complications of diabetes mellitus, such as neuropathy (*see* Clements *et al.*, "Neural abnormalities in myo-inositol metabolism in the streptozotocin-diabetic rat" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 460-464 (1994)) and nephropathy (*see* Rasch *et al.*, "Experimental diabetic nephropathy" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 392-397 (1994); Cohen, "Basement membrane metabolism in experimental diabetes" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 405-407 (1994); and Pinter *et al.*, "Functional manifestations of microangiopathy in experimental diabetes mellitus in the renal postglomerular circulation" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 410-413 (1994)).

Wistar rats of either sex weighing 200-250g were injected with streptozotocin (45mg/kg, i.p). After 48h blood was sampled from the tail vein and elevated plasma glucose determined by a glucose oxidation method. Animals had access to water ad libitum and were fed Purina rat chow (São Paulo-Brazil) up to 24h before surgery when food was removed but water was still permitted. Animals were then anaesthetized with sodium pentobarbital (50mg/kg), injected with propranolol (5mg/kg, i.p) to counteract sympathetic responses and a midline incision was made in the anterior cervical region. The external left jugular vein was identified, cannulated and prepared for drug infusion.

3-*O*-methyl-D-*chiro*-inositol (15mg/kg in 0.5ml 0.9% NaCl), DCI (15mg/kg in 0.5ml 0.9% NaCl) or 0.9% NaCl saline vehicle alone were injected into the jugular vein as a bolus.

In another group, the venous cannula was connected to an infusion pump (Buchler, Instruments Chicago , III) and manganese chloride, at a rate of 8.3mg/min were administered to the animals for a period of 2h.

The interaction between 3-*O*-methyl-D-*chiro*-inositol and DCI with manganese was also studied. First a primer of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg in 0.5ml 0.9% NaCl) or DCI (15mg/kg in 0.5ml 0.9% NaCl) was injected into the jugular vein as a bolus and an infusion of $MnCl_2$ (8.3mg/min) was coadministered during 2h. All the drugs and reagents were obtained

from Sigma. 3-*O*-methyl-D-*chiro*-inositol and DCI were obtained from another commercial source.

Before drug injection or infusion , a zero time sample (0.5ml) was taken from the tail vein and centrifuged. Clear sera were used for glucose determination by glucose oxidase. This was repeated every 20 min during 2h.

Statistical analysis

For each animal , the zero time value was set at 100%. The corresponding percentage for each time point was calculated for each animal and averaged. Group differences were compared, first by one way analysis of variance; those variables that had a significant F value were further tested by Student-Newman-Keuls. All data are expressed as the Mean ± SEM.

Results

A bolus dose of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) promoted a persistent hypoglycemic effect of 25% which was statistically different from saline group at 60, 80, 100 and 120 min ($p<0.05$) (Fig. 1).

Infusion of 8.3mg/min of manganese chloride lowered plasma glucose concetrations 23% at 120 min. This effect was achieved during the final 60 min and it was significantly different from saline group at 80, 100 and 120 min ($p<0.05$). The group treated with a primer of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) and a prolonged infusion of $MnCl_2$ (8.3mg/min) demonstrated a fall in plasma glucose concentrations of 49%. 3-*O*-methyl-D-*chiro*-inositol together with manganese reduced hyperglycemia to euglycemia (115 ± 07mg/dl) at 120 min. The rate of decline in plasma glucose of the group treated with 3-*O*-methyl-D-*chiro*-inositol plus manganese chloride was similar to that produced by 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) during the first 60min. When half of the solution of $MnCl_2$ was infused, the hypoglycemic effect with injected 3-*O*-methyl-D-*chiro*-inositol was potentiated. The effect of 3-*O*-methyl-D-*chiro*-inositol associated with manganese chloride was

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significantly different to that promoted by 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) at 120 min ($p<0.05$) (Fig. 1).

A bolus dose of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) promoted a 21% decrease in plasma glucose, which was statistically different from the saline control at 80, 100 and 120min ($p<0.05$). The coadministration of DCI (15mg/kg) and manganese chloride (8.3mg/min) reduced elevated blood glucose 47%. This hypoglycemic effect was significantly different to that produced by DCI (15mg/kg) at 120 min ($p<0.05$) (Fig.1).

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for improving glucose metabolism and/or increasing insulin sensitivity in a mammal, which comprises: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

2. A method for improving glucose metabolism in a mammal, which comprises the step of administering to said mammals (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

3. A method for increasing insulin sensitivity in a mammal, which comprises the step of administering to said mammal: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

4. A method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity, which comprises the step of administering to a mammal: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite thereof.

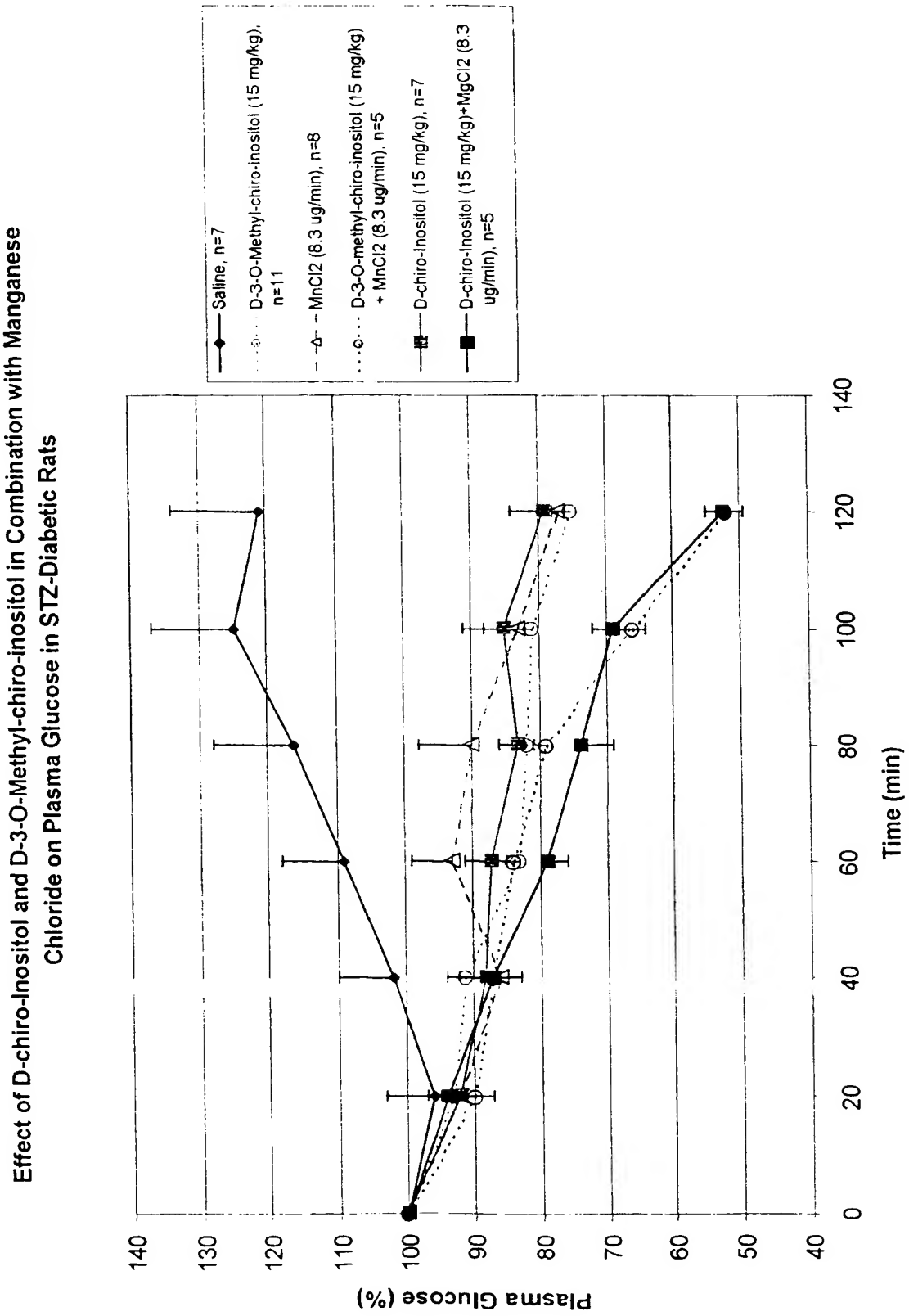

Figure 1. At 120 minutes, the hypoglycaemic effects produced by combination of either D-chiro-inositol or D-3-O-methyl-chiro-inositol with manganese chloride are significantly ($p<0.05$) greater than those produced by D-chiro-inositol or D-3-O-methyl-chiro-inositol alone.

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

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Published:
— *With international search report.*

(88) Date of publication of the international search report:
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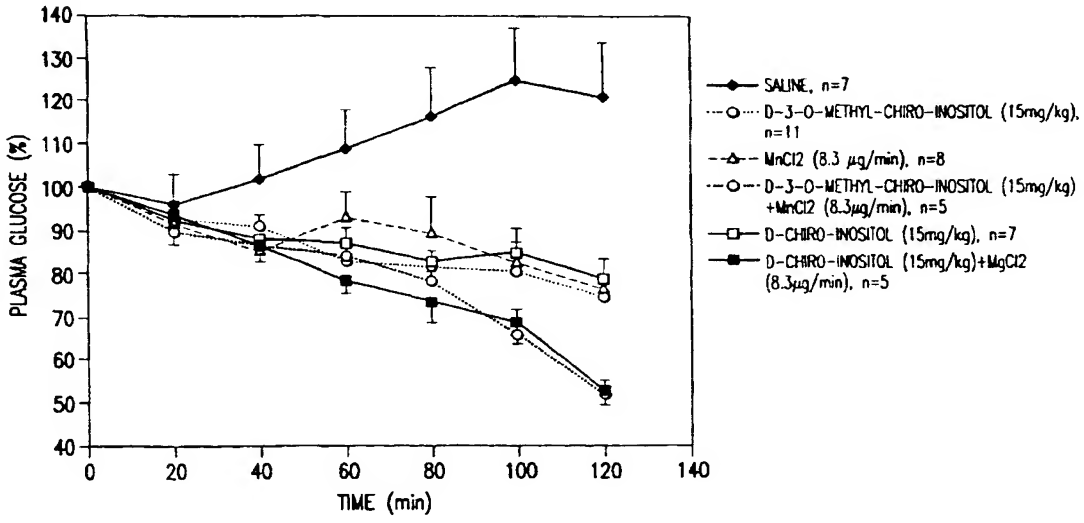
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING AN INOSITOL AND A METAL ION FOR IMPROVING INSULIN SENSITIVITY AND GLUCOSE METABOLISM



(57) Abstract: The current invention relates to compositions and methods for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. The compositions of the present invention contain (i) a source of a polyvalent metal ion, such as magnesium, manganese, chromium, vanadium and zinc, and (ii) an inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K33/32 A61K33/30 A61K33/24 A61K33/06 A61K31/045
A61P3/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, CANCERLIT, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 659 349 A (SQUIBB BRISTOL MYERS CO) 28 June 1995 (1995-06-28) page 7, line 1 -page 8, line 10 ---	1-4
X	US 5 308 627 A (UMBDENSTOCK JR ANTHONY J) 3 May 1994 (1994-05-03) claim 1 ---	1-4
X	GRAFTON G ET AL: "EFFECT OF MAGNESIUM ON SODIUM-DEPENDENT INOSITOL TRANSPORT ROLE FOR MAGNESIUM IN ETIOLOGY OF DIABETIC COMPLICATIONS." DIABETES, (1992) 41 (1), 35-39. , XP000949916 abstract -----	1-4

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2000	27/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Leherte, C

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4

Present claims 1-4 relate to compositions defined (inter alia) by reference to the following parameters:
P1: a source of a bivalent metal ion;
P2: an inositol or derivate or metabolite thereof.
The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compostions mentioned in the examples of the description at pages 18-22 , with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern al Application No
PCT/US 00/11196

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0659349 A	28-06-1995	AU 8163394 A	29-06-1995
		CA 2137431 A	23-06-1995
		JP 7223939 A	22-08-1995
		US 5763392 A	09-06-1998
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		AU 7353994 A	29-11-1995
		US 5332579 A	26-07-1994
		EP 0732917 A	25-09-1996
		JP 9504036 T	22-04-1997

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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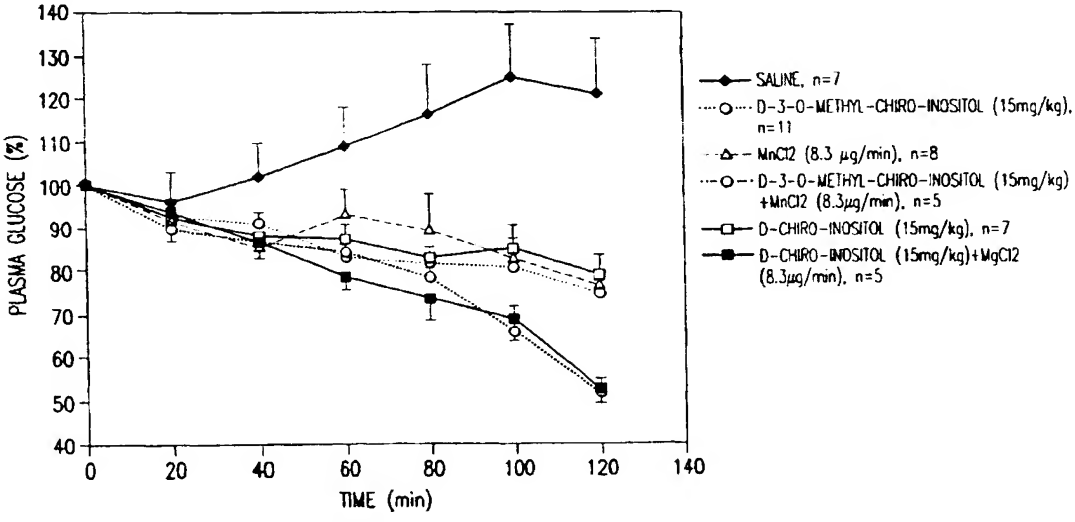
(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING AN INOSITOL AND A METAL ION FOR IMPROVING INSULIN SENSITIVITY AND GLUCOSE METABOLISM



(57) Abstract: The current invention relates to compositions and methods for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. The compositions of the present invention contain (i) a source of a polyvalent metal ion, such as magnesium, manganese, chromium, vanadium and zinc, and (ii) an inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette

PHARMACEUTICAL COMPOSITION COMPRISING AN INOSITOL AND A METAL ION FOR IMPROVING INSULIN SENSITIVITY AND GLUCOSE METABOLISM

FIELD OF THE INVENTION

The current invention relates to compositions and methods for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. The compositions of the present invention contain (i) a source of a polyvalent metal ion, and (ii) an inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol.

BACKGROUND OF THE INVENTION

A. INSULIN AND METABOLISM

In the mammalian body, insulin acts as a kind of spark plug to the mechanism that allows glucose in the blood to enter a cell, where it can be used as energy. Without insulin, sugar levels in the blood increase to dangerous and even life-threatening levels, a condition known in humans as diabetes mellitus. The supply of glucose to the cells is essential not only for energy, but also for the functioning of the mammalian brain and the survival of sensitive nerve tissue.

After food is consumed by a mammal, the pancreas secretes insulin into the bloodstream to maintain an optimal blood sugar level, generally about 4-8 mmol/L (about 72-144 mg/dL) in healthy humans. Other hormones, however, including glucagon, cortisone, adrenaline and growth hormone, act against insulin to prevent the blood sugar level from dropping too low.

When there is insufficient food-derived glucose in the blood to satisfy the needs of the mammalian brain, the body produces its own glucose, first through the breakdown of stored glycogen by the liver and then through gluconeogenesis. In gluconeogenesis, amino acids from food or muscle tissue are converted to glucose. Gluconeogenesis is inhibited by insulin.

A second source of fuel for the mammalian brain is ketone bodies, which are formed from fatty acids stored in adipose tissue. After an extended period of

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starvation or carbohydrate restriction, ketone bodies are formed by the body (ketogenesis) to supplement the glucose formed by the body through gluconeogenesis. Ketogenesis requires the influence of glucagon to proceed and is inhibited by insulin.

In addition to gluconeogenesis and ketogenesis, insulin also affects a number of other processes in the body. For example, insulin causes a buildup of triglycerides, the storage form of fatty acids, and inhibits their release from fat cells. Insulin also increases the formation of cholesterol in the liver and similar steroid hormones in the adrenals and gonads. Moreover, insulin favors the retention of water and salt by the mammalian body, thereby increasing blood pressure and aggravating hypertension.

B. INSULIN SENSITIVITY AND ABNORMAL GLUCOSE TOLERANCE

Insulin sensitivity is a measurement of insulin's ability to produce a biological response; specifically, in the case of glucose regulation, insulin sensitivity is a measurement of insulin's ability to promote the clearance and utilization of glucose.

A decrease in insulin sensitivity will result in a prolonged elevation of glucose levels and the release of additional insulin to try and achieve a euglycemic state. This compensatory hyperinsulinemia will effect insulin's ability to suppress lipolysis in adipose tissue, thus causing an increase in free fatty acids and ultimately resulting in the disruption of normal lipid profiles which could lead to coronary artery disease. The increase in free fatty acids will also inhibit insulin-stimulated glucose utilization in the muscle and stimulate hepatic gluconeogenesis. This leads to increased blood glucose and will eventually result in the development of impaired glucose tolerance or impaired fasting glucose and ultimately, if unchecked, the development of Type 2 diabetes. Improving insulin sensitivity will restore overall glucose control and decrease the risk of cardiovascular disease.

In mammals, a reduction in insulin sensitivity (an increase in insulin resistance) is accompanied by an increase in serum glucose concentration. This increase in serum glucose concentration, in turn, causes an increase in insulin production by the pancreas

in an effort to maintain normal blood glucose levels. Prolonged elevated serum insulin and glucose levels are therefore characteristic of insulin resistance.

Even though serum glucose levels remain high in insulin resistant mammals due to the high amounts of ineffective insulin, this insulin continues to function normally with respect to its other physiological roles. Thus, an insulin resistant person who already has high blood sugar levels, which normally should signal satisfaction, often experiences carbohydrate cravings, particularly for simple sugars. As more carbohydrates are consumed, the pancreas produces additional insulin to maintain a constant serum glucose concentration. That additional insulin, however, enhances the insulin resistance (decreases insulin sensitivity) by decreasing the number of available insulin receptors on the surfaces of the cells.

Abnormal glucose tolerance refers to metabolic stages intermediary to normal glucose homeostasis and Type 2 diabetes; this includes conditions like impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) where glucose values are above the conventional normal range and are often accompanied by a decrease in insulin sensitivity. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are transient, intermediate stages in the development of Type 2 diabetes. Within ten-years of diagnosis, approximately 30% of IGT subjects will progress to Type 2 diabetes and potentially to health problems that accompany this disease, including retinopathy, nephropathy, and peripheral neuropathy. In addition, abnormal glucose tolerance and decreased insulin sensitivity are associated with a high risk for the development of hypertension, dyslipidemia and an increase incidence of coronary artery disease.

Abnormal glucose tolerance and decreased insulin sensitivity can be attributed to a wide range of causes including obesity, age, physical activity level, certain medication or drugs, genetic factors, and some endocrine related disorders. The truncal distribution of weight as determined by a high waist to hip ratio (WHR) is a good predictor of abnormal insulin sensitivity, and there is an excellent correlation

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between a high body mass index (BMI) and decreased insulin sensitivity. Approximately 33% of the population in the United States is obese and the majority of these individuals have decreased insulin sensitivity, are hyperinsulinemic, and often have abnormal glucose tolerance.

Impaired fasting glucose (IFG) is defined by the American Diabetes Association as a fasting blood glucose in the range of 110 mg/dL to 125 mg/dL. IFG is determined by analysis of a plasma sample for glucose after a 10-16 hour fast. This is an easy and quick way to determine if there is a problem with glucose tolerance and metabolism.

Impaired glucose tolerance, as defined by the World Health Organization, is determined by the administration of a standard oral glucose tolerance test (OGTT) (World Health Org., Diabetes Mellitus, Tech. Rep. Ser., no. 727 (1985)). During an OGTT, a measured amount of glucose (75 grams) is given to a fasted individual and blood glucose levels are measured every 30 minutes, usually for 2 or 3 hours. In a individual with normal glucose tolerance, the blood glucose values will rise during the first part of the test and then rapidly return to basal levels. In an IGT individual the post prandial glucose levels will rise above the normal range, producing a 2-hour glucose value of 140-199 mg/dL, and return to basal levels at a slow rate.

Abnormal glucose tolerance is caused in part by inadequate utilization of glucose in the periphery - at the site of the muscles. In addition, high fasting glucose values, seen with impaired fasting glucose, suggest that hepatic glucose production is not being effectively regulated. The underlying cause of this abnormal glucose tolerance is characterized by a decrease in insulin sensitivity.

Improving insulin sensitivity and glucose tolerance will help delay and even prevent the onset of Type 2 diabetes mellitus, and thus improve the quality of life by preventing acute and long-term complications, reducing mortality and treating accompanying disorders of those at risk for Type 2 diabetes.

Accordingly, there is a need for compositions and methods for improving insulin sensitivity and glucose metabolism in mammals, particularly in human.

SUMMARY OF THE INVENTION

Administration of salts or complexes of certain polyvalent metal ions to mammalian subjects with disease states characterized by reduced insulin sensitivity and/or abnormal glucose tolerance has been shown to produce beneficial therapeutic effects on glucose metabolism. Similarly, administration of certain inositols has also been shown to produce beneficial therapeutic effects on glucose metabolism in such subjects.

It has now been found that certain combinations of: (i) a salt or complex of polyvalent metal ions; and (ii) an inositol or a derivative of inositol have been found to exert significant effects on mammalian endocrinology and metabolism. More specifically, it has now been found that administration of a combination of:

(i) a salt or complex of certain polyvalent metal ion, such as magnesium, manganese, chromium, vanadium and zinc; and

(ii) an isomer of inositol, such as D-*chiro*-inositol, or a derivative or metabolite of an inositol, such as D-3-*O*-methyl-*chiro*-inositol,

to mammalian subjects with reduced insulin sensitivity and/or abnormal glucose tolerance, including type 2 diabetics, significantly improves glucose metabolism and/or increases insulin sensitivity at a lower dose and/or to a greater degree than either the polyvalent metal ion or the inositol alone.

Accordingly, the first embodiment of the present invention is directed to a pharmaceutical composition for improving glucose metabolism and/or increasing insulin sensitivity in a mammal, the inventive composition comprising: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol or a derivative or metabolite of an inositol.

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A second embodiment of the present invention is directed to a method for improving glucose metabolism and or increasing insulin sensitivity in a mammal, which comprises the step of administering to a mammal: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol.

A third embodiment of the present invention is directed to a method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity, which comprises the step of administering to a mammal: (i) an effective amount of a source of a polyvalent metal ion; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 is a graph comparing the plasma glucose levels of diabetic rats (streptozotocin 45 mg/kg) treated with: (i) NaCl (—◆—, n=7); (ii) 15 mg/kg D-3-*O*-methyl-*chiro*-inositol (red --○--, n=11); (iii) 8.3 µg/min manganese chloride (red --△--, n=8); (iv) 15 mg/kg D-3-*O*-methyl-*chiro*-inositol plus 8.3 µg/min manganese chloride (--○--, n=5); (v) 15 mg/kg D-*chiro*-inositol (red —■—, n=7); and (vi) 15 mg/kg D-*chiro*-inositol plus 8.3 µg/min manganese chloride (—■—, n=5). The data show that after 120 minutes, the hypoglycaemic effects produced by the combination of a source of polyvalent metal ions (manganese chloride) and either an inositol (D-*chiro*-inositol) or an inositol derivative (D-3-*O*-methyl-*chiro*-inositol) are significantly ($p<0.05$) greater than the effects produced by any of these agents alone.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In a first preferred embodiment, the present invention is directed to compositions for increasing insulin sensitivity and/or improving glucose metabolism in mammals, particularly humans. These mammals may have diabetes, particularly type 2 diabetes (insulin resistant diabetes), or may have only impaired glucose tolerance and have not yet developed diabetes.

In a second preferred embodiment, the present invention is directed to a method for improving glucose metabolism and/or increasing insulin sensitivity in mammals, particularly humans. These mammals may have diabetes, particularly type 2 diabetes (insulin resistant diabetes), or may have only impaired glucose tolerance and have not yet developed diabetes.

In a third preferred embodiment, the present invention is directed to a method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity. These mammalian metabolic diseases include, but are not limited to, the following: diabetes mellitus and its chronic complications; gestational diabetes; pre-eclampsia; obesity; hyperlipidemia and/or dyslipidemia; atherosclerosis; hypertension; cardiovascular disease; AIDS; cancer; wasting and/or cachexia; sepsis; trauma, such as associated with burns, malnutrition and/or stress; aging; autoimmune diseases, such as lupus; endocrine diseases; hyperuricemia; polycystic ovary disease; and complications arising from athletic activity or inactivity.

The compositions of the present invention, which are particularly useful in the therapeutic methods of the present invention, comprise: (i) an effective amount of a source of polyvalent metal ions; and (ii) an effective amount of an inositol, or a derivative or metabolite of an inositol. The inventive compositions may also optionally contain one or more pharmaceutically acceptable carriers, diluents and/or excipients.

The polyvalent metal may be any of the known metal elements which have at least one valence or oxidation state of 2 or greater. Thus, the free ions of these polyvalent metals have a positive charge of 2 or more. Illustrative examples of suitable polyvalent metals include, but are not limited to, the following metals: manganese; magnesium; chromium; zinc; vanadium; copper; titanium; and calcium.

Preferably, the polyvalent metal is a divalent metal, *i.e.*, has a valence or oxidation state of 2. Illustrative examples of suitable divalent metals include, but are not limited to, the following: manganese, magnesium, chromium, vanadium and zinc. Some polyvalent metals, such as chromium, exhibit more than one valence or oxidation state. In such instances, it is preferred that the polyvalent metal be used in its divalent state.

The source of polyvalent metal ions in the inventive compositions may be any material that will liberate the desired free polyvalent metal ions in the desired environment, such as in a mammalian body. Suitable sources of polyvalent metal ions include, but are not limited to, the following: polyvalent metal salts, such as magnesium chloride or manganese sulfate; and polyvalent metal chelates, such as magnesium EDTA (ethylene diamine tetraacetic acid).

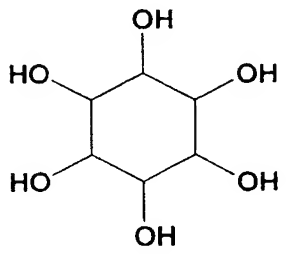
Preferably, the source of polyvalent metal ions is a salt of the desired polyvalent metal and one or more organic or inorganic counterions. Illustrative examples of suitable organic counterions include, but are not limited to, the following: maleate; acetate; tartrate; bitartrate; citrate; oxalate; succinate; benzoate; fumarate; malate; and mandelate. Illustrative examples of suitable inorganic counterions include, but are not limited to, the following: fluoride; chloride; bromide; iodide; sulfite; sulfate; sulfamate; nitrite; nitrate; phosphite; and phosphate.

Particularly preferred counterions are inorganic counterions; more particularly preferred counterions are halides, such as fluoride and chloride; most preferably, the counterion is chloride. Most preferably, the polyvalent metal ion is magnesium or manganese and the source of polyvalent metal ions is the chloride salt thereof.

Optionally, the inventive composition may contain more than one source of polyvalent metal ions. For example, a composition according to the present invention may include both magnesium chloride and magnesium sulfate in admixture.

Additionally, the inventive composition may optionally contain sources of more than one polyvalent metal ion. For example, a composition according to the present invention may include both magnesium chloride and manganese chloride in admixture.

The inositol employed in the inventive compositions may be any of the known isomers of inositol, a carbocyclic sugar having the general formula:



Illustrative examples of suitable isomers of inositol include, but are not limited to, the following: *myo*-inositol and *chiro*-inositol. Preferably, the isomer of inositol is *chiro*-inositol, more preferably D-*chiro*-inositol.

As used herein, a "derivative or metabolite of an inositol" may be any compound based on or derived from or containing a D-*chiro*-inositol moiety. Illustrative examples of suitable derivatives and metabolites of D-*chiro*-inositol include, but are not limited to, the following: D-*chiro*-inositol phosphates; D-*chiro*-inositol esters, preferably acetates; D-*chiro*-inositol ethers, preferably lower alkyl ethers; D-*chiro*-inositol acetals; D-*chiro*-inositol ketals; and compounds containing D-*chiro*-inositol.

As used herein, a "compound containing D-*chiro*-inositol" may be any compound that contains the D-*chiro*-inositol moiety. Illustrative examples of compounds containing D-*chiro*-inositol include, but are not limited to, the following:

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polysaccharides containing D-*chiro*-inositol and one or more additional sugars, such as glucose, galactose and mannose, or derivatives thereof, such as glucosamine, galactosamine and mannitol; D-*chiro*-inositol phospholipids; and complexes or chelates of D-*chiro*-inositol with one or more metal ions and the like.

Optionally, the inventive composition may contain both an inositol and a derivative or metabolite of an inositol. For example, a composition according to the present invention may include both D-*chiro*-inositol and D-3-*O*-methyl-*chiro*-inositol in admixture.

The compositions of the present invention will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the active agent), the site of delivery of the composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The"effective amount" of each active agent (*i.e.* (i) a source of polyvalent metal ions, and (ii) an inositol, or a derivative or metabolite thereof) for the purposes of the present invention is determined in view of such considerations. Those skilled in the art can readily determine empirically an appropriate "effective amount" of each active agent for a particular mammalian patient.

The key factor in selecting an appropriate dose is, of course, the desired result obtained in terms of improving glucose metabolism and/or increasing sensitivity. These desired results may be measured, for example, by increases or decreases in blood glucose levels and/or insulin sensitivity in the patient. The length of treatment needed to observe changes and the interval following treatment for responses to occur may vary depending on the desired effect and the particular patient, but may be determined empirically by those skilled in the art.

As a general proposition, the total effective amount of each active agent administered per dose will be in the range of about 0.1 μg/kg/day to 100 mg/kg/day of mammalian patient body weight, although, as noted above, this will be subject to

therapeutic discretion. More preferably, for humans, the amount per dose is between about 10 μg/day and 2 g/day.

For example, when administered orally, the inventive composition preferably contains from about 1 mg to about 1200 mg of the inositol, or the derivative or metabolite of an inositol. In the case where the inositol is D-*chiro*-inositol, the inventive composition preferably contains from about 10 mg to about 900 mg of DCI, more preferably about 30 mg to about 600 mg of DCI, and most preferably about 100 mg to about 300 mg of DCI. In the case where the derivative or metabolite of inositol is D-3-*O*-methyl-*chiro*-inositol, the inventive composition preferably contains from about 10 mg to about 900 mg of D-3-*O*-methyl-*chiro*-inositol, more preferably about 30 mg to about 600 mg of D-3-*O*-methyl-*chiro*-inositol, and most preferably about 100 mg to about 300 mg of D-3-*O*-methyl-*chiro*-inositol.

As used herein, the phrase "pharmaceutically acceptable" is intended to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, a "pharmaceutically acceptable carrier" is a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the active agents of the inventive compositions from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Some illustrative examples of materials which can serve as pharmaceutically-acceptable carriers include, but are not limited to, the following: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl

cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the inventive pharmaceutical compositions.

Illustrative examples of pharmaceutically acceptable antioxidants include, but are not limited to, the following: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA),butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredients which can be combined with a carrier material to produce a single dosage form will generally be that amount of each active

ingredient which, together, produce the desired therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredients, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association at least one source of polyvalent metal ions and at least one inositol (or derivative or metabolite of an inositol) with the carrier and, optionally, one or more accessory ingredients.

In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients the inventive compositions with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of each active ingredient. The active ingredients of the inventive compositions may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredients are mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption

accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents.

In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding the active ingredients and carrier material(s), optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredients moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredients can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the inventive compositions include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredients, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active ingredients, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing the active ingredients of the present invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and

release the active ingredients. Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of the inventive compositions include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredients may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to the active ingredients, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active ingredients, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active ingredients in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredients compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredients in a polymer matrix or gel. Devices, including patches, which transdermally deliver the active ingredients by ionophoresis or other electrically-assisted methods can also be employed in the present invention, including, for example, the devices described in U.S. Patent Nos. 4,708,716 and 5,372,579.

Ophthalmic formulations, eye ointments, powders, solutions, drops, sprays and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise at least one source of polyvalent metal ions and at least one inositol (or derivative or metabolite thereof) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Illustrative examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include, but are not limited to, the following: water; ethanol; polyols, such as glycerol, propylene glycol, polyethylene glycol, and the like, and suitable mixtures thereof; vegetable oils, such as olive oil; and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorbutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material

having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject active ingredients in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.01 to 99.5% (more preferably, 0.1 to 90%) of each active ingredient together in combination with at least one pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc.; administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is particularly preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein are intended to mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular,

intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein are intended to mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The inventive compositions may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the active ingredients of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

As noted, actual dosage levels of the active ingredients in the inventive pharmaceutical compositions may be varied so as to obtain an amount of each active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors, including, but not limited to, the following: the activity of the particular polyvalent metal ions and the particular inositol (or derivative or metabolite) employed; the route of administration; the time of administration; the rates of absorption, distribution, metabolism and/or excretion of the particular active ingredients being employed; the duration of the treatment; other drugs, compounds and/or materials used in combination with the particular active ingredients employed; the age, sex, weight,

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condition, general health and prior medical history of the patient being treated; and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine the effective amount of the each active ingredient required in the inventive pharmaceutical compositions. For example, the physician or veterinarian could start doses of the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

If desired, the effective daily dose of the active ingredients may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for the active ingredients of the present invention to be administered alone, it is preferable to administer these compounds as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, a therapeutic composition of the present invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,.486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an

osmotic drug delivery system. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

The following examples are illustrative only and are not intended to limit the scope of the invention as defined by the appended claims. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit and scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

All patents and publications referred to herein are hereby expressly incorporated in their entirety by reference.

EXAMPLES

Materials and methods

The streptozotocin (STZ) treated rat is a widely accepted and frequently used animal model of diabetes mellitus. At low doses of STZ, rats develop mild basal hyperglycemia, glucose intolerance and impaired glucose-induced insulin secretion. Therefore, this model appears to be an excellent model of human type 2 (non-insulin dependent) diabetes and well-suited for the study of novel antidiabetic agents (*see* Pele-Tounian *et al.*, *British J. Pharmacol. 124*:1591-1596 (1998)). At larger doses of STZ, absolute insulinopenia and extreme levels of glycemia develop, and the diabetes becomes life-threatening similar to human type 1 (juvenile onset) diabetes.

In addition to being an excellent model of aberrant insulin and glucose metabolism in humans, the STZ-treated diabetic rat is a widely used model of the severe and life-threatening long-term complications of diabetes mellitus, such as neuropathy (*see* Clements *et al.*, "Neural abnormalities in myo-inositol metabolism in

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the streptozotocin-diabetic rat" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 460-464 (1994)) and nephropathy (*see* Rasch *et al.*, "Experimental diabetic nephropathy" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 392-397 (1994); Cohen, "Basement membrane metabolism in experimental diabetes" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 405-407 (1994); and Pinter *et al.*, "Functional manifestations of microangiopathy in experimental diabetes mellitus in the renal postglomerular circulation" in *Lessons from Animal Diabetes*, Shafrir *et al.*, eds., pp. 410-413 (1994)).

Wistar rats of either sex weighing 200-250g were injected with streptozotocin (45mg/kg, i.p). After 48h blood was sampled from the tail vein and elevated plasma glucose determined by a glucose oxidation method. Animals had access to water ad libitum and were fed Purina rat chow (São Paulo-Brazil) up to 24h before surgery when food was removed but water was still permitted. Animals were then anaesthetized with sodium pentobarbital (50mg/kg), injected with propranolol (5mg/kg, i.p) to counteract sympathetic responses and a midline incision was made in the anterior cervical region. The external left jugular vein was identified, cannulated and prepared for drug infusion.

3-*O*-methyl-D-*chiro*-inositol (15mg/kg in 0.5ml 0.9% NaCl), DCI (15mg/kg in 0.5ml 0.9% NaCl) or 0.9% NaCl saline vehicle alone were injected into the jugular vein as a bolus.

In another group, the venous cannula was connected to an infusion pump (Buchler, Instruments Chicago , III) and manganese chloride, at a rate of 8.3mg/min were administered to the animals for a period of 2h.

The interaction between 3-*O*-methyl-D-*chiro*-inositol and DCI with manganese was also studied. First a primer of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg in 0.5ml 0.9% NaCl) or DCI (15mg/kg in 0.5ml 0.9% NaCl) was injected into the jugular vein as a bolus and an infusion of $MnCl_2$ (8.3mg/min) was coadministered during 2h. All

the drugs and reagents were obtained from Sigma. 3-*O*-methyl-D-*chiro*-inositol and DCI were obtained from another commercial source.

Before drug injection or infusion , a zero time sample (0.5ml) was taken from the tail vein and centrifuged. Clear sera were used for glucose determination by glucose oxidase. This was repeated every 20 min during 2h.

Statistical analysis

For each animal , the zero time value was set at 100%. The corresponding percentage for each time point was calculated for each animal and averaged. Group differences were compared, first by one way analysis of variance; those variables that had a significant F value were further tested by Student-Newman-Keuls. All data are expressed as the Mean ± SEM.

Results

A bolus dose of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) promoted a persistent hypoglycemic effect of 25% which was statistically different from saline group at 60, 80, 100 and 120 min ($p<0.05$) (Fig. 1).

Infusion of 8.3mg/min of manganese chloride lowered plasma glucose concetrations 23% at 120 min. This effect was achieved during the final 60 min and it was significantly different from saline group at 80, 100 and 120 min ($p<0.05$). The group treated with a primer of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) and a prolonged infusion of $MnCl_2$ (8.3mg/min) demonstrated a fall in plasma glucose concentrations of 49%. 3-*O*-methyl-D-*chiro*-inositol together with manganese reduced hyperglycemia to euglycemia (115 ± 07mg/dl) at 120 min. The rate of decline in

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plasma glucose of the group treated with 3-*O*-methyl-D-*chiro*-inositol plus manganese chloride was similar to that produced by 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) during the first 60min. When half of the solution of $MnCl_2$ was infused, the hypoglycemic effect with injected 3-*O*-methyl-D-*chiro*-inositol was potentiated. The effect of 3-*O*-methyl-D-*chiro*-inositol associated with manganese chloride was significantly different to that promoted by 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) at 120 min ($p<0.05$) (Fig. 1).

A bolus dose of 3-*O*-methyl-D-*chiro*-inositol (15mg/kg) promoted a 21% decrease in plasma glucose, which was statistically different from the saline control at 80, 100 and 120min ($p<0.05$). The coadministration of DCI (15mg/kg) and manganese chloride (8.3mg/min) reduced elevated blood glucose 47%. This hypoglycemic effect was significantly different to that produced by DCI (15mg/kg) at 120 min ($p<0.05$) (Fig.1).

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for improving glucose metabolism and/or increasing insulin sensitivity in a mammal, which comprises: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

2. A method for improving glucose metabolism in a mammal, which comprises the step of administering to said mammals (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

3. A method for increasing insulin sensitivity in a mammal, which comprises the step of administering to said mammal: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite of an inositol.

4. A method for treating mammalian metabolic diseases characterized by abnormal glucose metabolism and/or decreased insulin sensitivity, which comprises the step of administering to a mammal: (i) an effective amount of at least one source of a polyvalent metal ion; and (ii) an effective amount of at least one inositol, or a derivative or metabolite thereof.

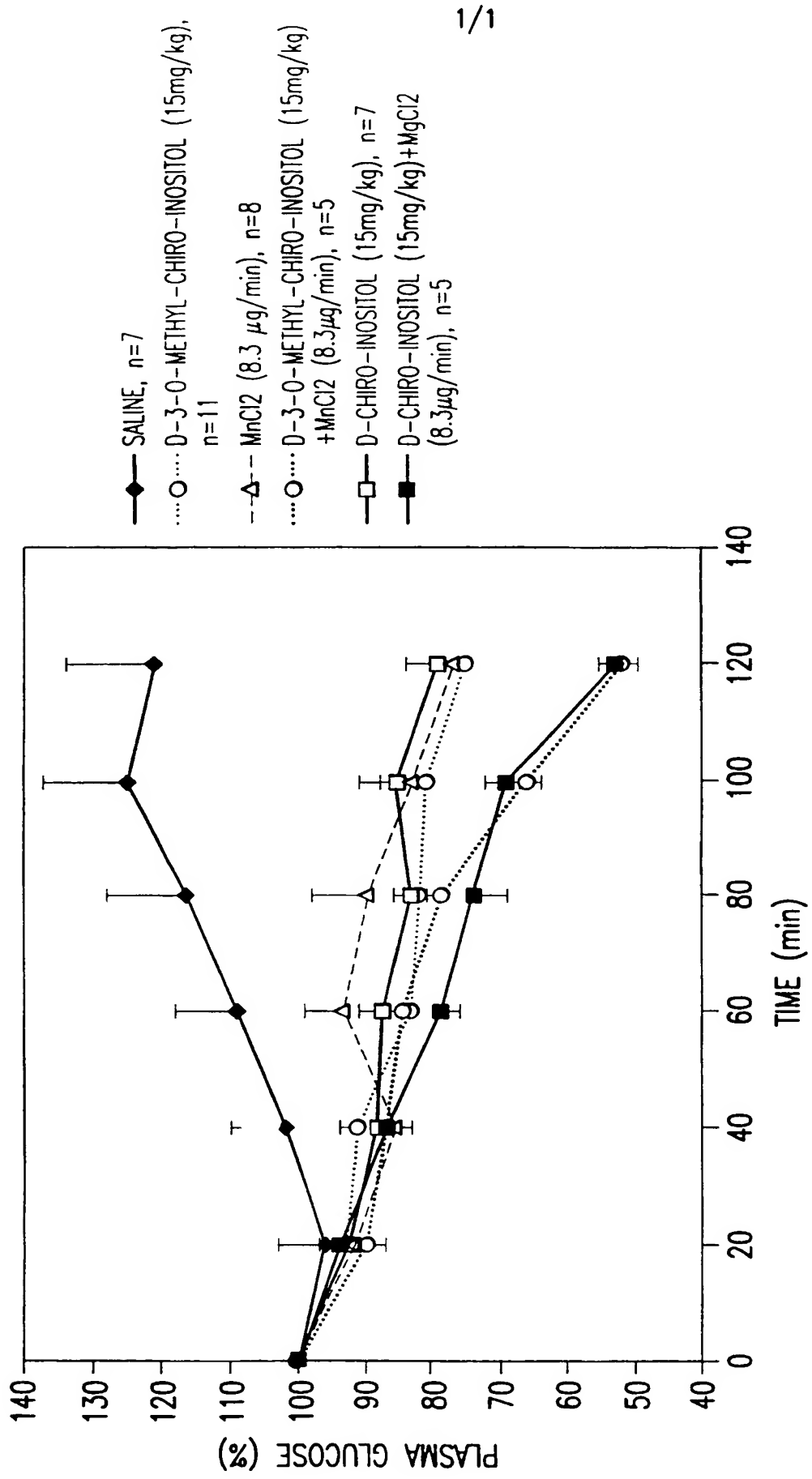
SALINE, n=7
D-3-O-METHYL-CHIRO-INOSITOL (15mg/kg), n=11
MnCl2 (8.3 μg/min), n=8
D-3-O-METHYL-CHIRO-INOSITOL (15mg/kg) +MnCl2 (8.3μg/min), n=5
D-CHIRO-INOSITOL (15mg/kg), n=7
D-CHIRO-INOSITOL (15mg/kg)+MgCl2 (8.3μg/min), n=5
PLASMA GLUCOSE (%)
TIME (min)
140
130
120
110
100
90
80
70
60
50
40
0
20
40
60
80
100
120
140

FIG.1

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/US 00/11196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K33/32 A61K33/30 A61K33/24 A61K33/06 A61K31/045
A61P3/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, CANCERLIT, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 659 349 A (SQUIBB BRISTOL MYERS CO) 28 June 1995 (1995-06-28) page 7, line 1 -page 8, line 10 ---	1-4
X	US 5 308 627 A (UMBDENSTOCK JR ANTHONY J) 3 May 1994 (1994-05-03) claim 1 ---	1-4
X	GRAFTON G ET AL: "EFFECT OF MAGNESIUM ON SODIUM-DEPENDENT INOSITOL TRANSPORT ROLE FOR MAGNESIUM IN ETIOLOGY OF DIABETIC COMPLICATIONS." DIABETES, (1992) 41 (1), 35-39. , XP000949916 abstract -----	1-4

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2000	27/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (July 1992)

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4

Present claims 1-4 relate to compositions defined (inter alia) by reference to the following parameters:
P1: a source of a bivalent metal ion;
P2: an inositol or derivate or metabolite thereof.
The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compostions mentioned in the examples of the description at pages 18-22 , with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

...rmation on patent family members

Intern ...al Application No
PCT/US 00/11196

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0659349 A	28-06-1995	AU 8163394 A	29-06-1995
		CA 2137431 A	23-06-1995
		JP 7223939 A	22-08-1995
		US 5763392 A	09-06-1998
US 5308627 A	03-05-1994	WO 9529668 A	09-11-1995
		AU 7353994 A	29-11-1995
		US 5332579 A	26-07-1994
		EP 0732917 A	25-09-1996
		JP 9504036 T	22-04-1997

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